TITLE: HYPERSENSITIVE RESPONSE INDUCED RESISTANCE
        IN PLANTS BY SEED TREATMENT

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HYPERSENSITIVE RESPONSE INDUCED RESISTANCE
IN PLANTS BY SEED TREATMENT

5   This application claims the benefit of U.S. Provisional Patent Application Serial No. 60/033,230, filed December 5, 1996.

FIELD OF THE INVENTION

       The present invention relates to imparting
15   hypersensitive response induced resistance to plants by treatment of seeds.

BACKGROUND OF THE INVENTION

20   Living organisms have evolved a complex array of biochemical pathways that enable them to recognize and respond to signals from the environment. These pathways include receptor organs, hormones, second messengers, and enzymatic modifications. At present, little is known
25   about the signal transduction pathways that are activated during a plant's response to attack by a pathogen, although this knowledge is central to an understanding of disease susceptibility and resistance. A common form of plant resistance is the restriction of pathogen
30   proliferation to a small zone surrounding the site of infection. In many cases, this restriction is accompanied by localized death (i.e., necrosis) of host tissues. Together, pathogen restriction and local tissue necrosis characterize the hypersensitive response. In
35   addition to local defense responses, many plants respond to infection by activating defenses in uninfected parts of the plant. As a result, the entire plant is more resistant to a secondary infection. This systemic

- 2 -

acquired resistance can persist for several weeks or more
(R.E.F. Matthews, Plant Virology (Academic Press, New
York, ed. 2, 1981)) and often confers cross-resistance to
unrelated pathogens (J. Kuc, in Innovative Approaches to
5 Plant Disease Control, I. Chet, Ed. (Wiley, New York,
1987), pp. 255-274, which is hereby incorporated by
reference). See also Kessman, et al., "Induction of
Systemic Acquired Disease Resistance in Plants By
Chemicals," Ann. Rev. Phytopathol. 32:439-59 (1994),
10 Ryals, et al., "Systemic Acquired Resistance," The Plant
Cell 8:1809-19 (Oct. 1996), and Neuenschwander, et al.,
"Systemic Acquired Resistance," Plant-Microbe
Interactions vol. 1, G. Stacey, et al. ed. pp. 81-106
(1996), which are hereby incorporated by reference.
15   Expression of systemic acquired resistance is
associated with the failure of normally virulent
pathogens to ingress the immunized tissue (Kuc, J.,
"Induced Immunity to Plant Disease," Bioscience, 32:854-
856 (1982), which is hereby incorporated by reference).
20 Establishment of systemic acquired resistance is
correlated with systemic increases in cell wall
hydroxyproline levels and peroxidase activity (Smith,
J.A., et al., "Comparative Study of Acidic Peroxidases
Associated with Induced Resistance in Cucumber, Muskmelon
25 and Watermelon," Physiol. Mol. Plant Pathol. 14:329-338
(1988), which is hereby incorporated by reference) and
with the expression of a set of nine families of
so-called systemic acquired resistance gene (Ward, E.R.,
et al., "Coordinate Gene Activity in Response to Agents
30 that Induce Systemic Acquired Resistance," Plant Cell
3:49-59 (1991), which is hereby incorporated by
reference). Five of these defense gene families encode
pathogenesis-related proteins whose physiological
functions have not been established. However, some of
35 these proteins have antifungal activity in vitro (Bol,

J.F., et al., "Plant Pathogenesis-Related Proteins Induced by Virus Infection," Ann. Rev. Phytopathol. 28:113-38 (1990), which is hereby incorporated by reference) and the constitutive expression of a bean

5   chitinase gene in transgenic tobacco protects against infection by the fungus *Rhizoctonia solani* (Broglie, K., et al., "Transgenic Plants with Enhanced Resistance to the Fungal Pathogen Rhizoctonia Solani," Science 254:1194-1197 (1991), which is hereby incorporated by

10   reference), suggesting that these systemic acquired resistance proteins may contribute to the immunized state (Uknes, S., et al., "Acquired Resistance in *Arabidopsis*," Plant Cell 4:645-656 (1992), which is hereby incorporated by reference).

15   Salicylic acid appears to play a signal function in the induction of systemic acquired resistance since endogenous levels increase after immunization (Malamy, J., et al., "Salicylic Acid: A Likely Endogenous Signal in the Resistance Response of Tobacco

20   to Viral Infection," Science 250:1002-1004 (1990), which is hereby incorporated by reference) and exogenous salicylate induces systemic acquired resistance genes (Yalpani, N., et al., "Salicylic Acid is a Systemic Signal and an Inducer of Pathogenesis-Related Proteins in

25   Virus-Infected Tobacco," Plant Cell 3:809-818 (1991), which is hereby incorporated by reference), and acquired resistance (Uknes, S., et al., "Acquired Resistance in *Arabidopsis*," Plant Cell 4:645-656 (1992), which is hereby incorporated by reference). Moreover, transgenic

30   tobacco plants in which salicylate is destroyed by the action of a bacterial transgene encoding salicylate hydroxylase do not exhibit systemic acquired resistance (Gaffney, T., et al., "Requirement of Salicylic Acid for the Induction of Systemic Acquired Resistance," Science

35   261:754-56 (1993), which is hereby incorporated by

reference). However, this effect may reflect inhibition of a local rather than a systemic signal function, and detailed kinetic analysis of signal transmission in cucumber suggests that salicylate may not be essential for long-distance signaling (Rasmussen, J.B., et al., "Systemic Induction of Salicylic Acid Accumulation in Cucumber after Inoculation with *Pseudomonas Syringae* pv. *Syringae*," Plant Physiol. 97:1342-1347) (1991), which is hereby incorporated by reference).

Immunization using biotic agents has been extensively studied. Green beans were systemically immunized against disease caused by cultivar-pathogenic races of *Colletotrichum lindemuthianum* by prior infection with either cultivar-nonpathogenic races (Rahe, J.E., "Induced Resistance in *Phaseolus Vulgaris* to Bean Anthracnose," Phytopathology 59:1641-5 (1969); Elliston, J., et al., "Induced Resistance to Anthracnose at a Distance from the Site of the Inducing Interaction," Phytopathology 61:1110-12 (1971); Skipp, R., et al., "Studies on Cross Protection in the Anthracnose Disease of Bean," Physiological Plant Pathology 3:299-313 (1973), which are hereby incorporated by reference), cultivar-pathogenic races attenuated by heat in host tissue prior to symptom appearance (Rahe, J.E., et al., "Metabolic Nature of the Infection-Limiting Effect of Heat on Bean Anthracnose," Phytopathology 60:1005-9 (1970), which is hereby incorporated by reference) or nonpathogens of bean. The anthracnose pathogen of cucumber, *Colletotrichum lagenarium*, was equally effective as non-pathogenic races as an inducer of systemic protection against all races of bean anthracnose. Protection was induced by *C. lagenarium* in cultivars resistant to one or more races of *C. lindemuthianum* as well as in cultivars susceptible to all reported races of the fungus and which accordingly had

been referred to as 'lacking genetic resistance' to the
pathogen (Elliston, J., et al., "Protection of Bean
Against Anthracnose by *Colletotrichum* Species
Nonpathogenic on Bean," Phytopathologische Zeitschrift
86:117-26 (1976); Elliston, J., et al., "A Comparative
Study on the Development of Compatible, Incompatible and
Induced Incompatible Interactions Between *Collectotrichum*
Species and *Phaseolus Vulgaris*," Phytopathologische
Zeitschrift 87:289-303 (1976), which are hereby
incorporated by reference). These results suggest that
the same mechanisms may be induced in cultivars reported
as 'possessing' or 'lacking' resistance genes (Elliston,
J., et al., "Relation of Phytoalexin Accumulation to
Local and Systemic Protection of Bean Against
Anthracnose," Phytopathologische Zeitschrift 88:114-30
(1977), which is hereby incorporated by reference). It
also is apparent that cultivars susceptible to all races
of *C. lindemuthianum* do not lack genes for induction of
resistance mechanisms against the pathogen.

Kuc, J., et al., "Protection of Cucumber
Against *Collectotrichum Lagenarium* by *Colletotrichum
Lagenarium*," Physiological Plant Pathology 7:195-9
(1975), which is hereby incorporated by reference),
showed that cucumber plants could be systemically
protected against disease caused by *Colletotrichum
lagenarium* by prior inoculation of the cotyledons or the
first true leaf with the same fungus. Subsequently,
cucumbers have been systemically protected against
fungal, bacterial, and viral diseases by prior localized
infection with either fungi, bacteria, or viruses
(Hammerschmidt, R., et al., "Protection of Cucumbers
Against *Colletotrichum Lagenarium* and *Cladosporium
Cucumerinum*," Phytopathology 66:790-3 (1976); Jenns, A.
E., et al., "Localized Infection with Tobacco Necrosis
Virus Protects Cucumber Against *Colletotrichum*

Lagenarium," <u>Physiological Plant Pathology</u> 11:207-12
(1977); Caruso, F.L., et al. "Induced Resistance of
Cucumber to Anthracnose and Angular Leaf Spot by
Pseudomonas Lachrymans and *Colletotrichum Lagenarium*,"

5   <u>Physiological Plant Pathology</u> 14:191-201 (1979); Staub,
T., et al., "Systemic Protection of Cucumber Plants
Against Disease Caused by *Cladosporium Cucumerinum* and
Colletotrichum Lagenarium by Prior Localized Infection
with Either Fungus," <u>Physiological Plant Pathology</u>,

10   17:389-93 (1980); Bergstrom, G.C., et al., "Effects of
Local Infection of Cucumber by *Colletotrichum Lagenarium*,
Pseudomonas Lachrymans or Tobacco Necrosis Virus on
Systemic Resistance to Cucumber Mosaic Virus,"
<u>Phytopathology</u> 72:922-6 (1982); Gessler, C., et al.,

15   "Induction of Resistance to *Fusarium* Wilt in Cucumber by
Root and Foliar Pathogens," <u>Phytopathology</u> 72:1439-41
(1982); Basham, B., et al., "Tobacco Necrosis Virus
Induces Systemic Resistance in Cucumbers Against
Sphaerotheca Fuliginea," <u>Physiological Plant Pathology</u>

20   23:137-44 (1983), which are hereby incorporated by
reference). Non-specific protection induced by infection
with *C. lagenarium* or tobacco necrosis virus was
effective against at least 13 pathogens, including
obligatory and facultative parasitic fungi, local lesion

25   and systemic viruses, wilt fungi, and bacteria.
Similarly, protection was induced by and was also
effective against root pathogens. Other curcurbits,
including watermelon and muskmelon have been systemically
protected against *C. lagenarium* (Caruso, F.L., et al.,

30   "Protection of Watermelon and Muskmelon Against
Colletotrichum Lagenarium by *Colletotrichum Lagenarium*,"
<u>Phytopathology</u> 67:1285-9 (1977), which is hereby
incorporated by reference).

Systemic protection in tobacco has also been

35   induced against a wide variety of diseases (Kuc, J., et

al., "Immunization for Disease Resistance in Tobacco,"
Recent Advances in Tobacco Science 9:179-213 (1983),
which is hereby incorporated by reference). Necrotic
lesions caused by tobacco mosaic virus enhanced

5   resistance in the upper leaves to disease caused by the
virus (Ross, A.F., et al., "Systemic Acquired Resistance
Induced by Localized Virus Infections in Plants,"
Virology 14:340-58 (1961); Ross, A.F., et al., "Systemic
Effects of Local Lesion Formation," In: Viruses of Plants

10   pp. 127-50 (1966), which are hereby incorporated by
reference). Phytophthora parasitica var. nicotianae, P.
tabacina and Pseudomonas tabaci and reduced reproduction
of the aphid Myzus persicae (McIntyre, J.L., et al.,
"Induction of Localized and Systemic Protection Against

15   Phytophthora Parasitica var. nicotianae by Tobacco Mosaic
Virus Infection of Tobacco Hypersensitive to the Virus,"
Physiological Plant Pathology 15:321-30 (1979); McIntyre,
J.L., et al., "Effects of Localized Infections of
Nicotiana Tabacum by Tobacco Mosaic Virus on Systemic

20   Resistance Against Diverse Pathogens and an Insect,"
Phytopathology 71:297-301 (1981), which are hereby
incorporated by reference). Infiltration of heat-killed
Pseudomonas tabacin (Lovrekovich, L., et al., "Induced
Reaction Against Wildfire Disease in Tobacco Leaves

25   Treated with Heat-Killed Bacteria," Nature 205:823-4
(1965), which is hereby incorporated by reference), and
Pseudomonas solanacearum (Sequeira, L, et al.,
"Interaction of Bacteria and Host Cell Walls: Its
Relation to Mechanisms of Induced Resistance,"

30   Physiological Plant Pathology 10:43-50 (1977), which is
hereby incorporated by reference), into tobacco leaves
induced resistance against the same bacteria used for
infiltration. Tobacco plants were also protected by the
nematode Pratylenchus penetrans against P. parasitica

35   var. nicotiana (McIntyre, J.L., et al. "Protection of

Tobacco Against *Phytophthora Parasitica* Var. *Nicotianae*
by Cultivar-Nonpathogenic Races, Cell-Free Sonicates and
Pratylenchus Penetrans," Phytopathology 68:235-9 (1978),
which is hereby incorporated by reference).

5   Cruikshank, I.A.M., et al., "The Effect of Stem
Infestation of Tobacco with *Peronospora Tabacina* Adam on
Foliage Reaction to Blue Mould," Journal of the
Australian Institute of Agricultural Science 26:369-72
(1960), which is hereby incorporated by reference, were
10   the first to report immunization of tobacco foliage
against blue mould (i.e., *P. tabacina*) by stem injection
with the fungus, which also resulted in dwarfing and
premature senescence. It was recently discovered that
injection external to the xylem not only alleviated
15   stunting but also promoted growth and development.
Immunized tobacco plants, in both glasshouse and field
experiments, were approximately 40% taller, had a 40%
increase in dry weight, a 30% increase in fresh weight,
and 4-6 more leaves than control plants (Tuzun, S., et
20   al., "The Effect of Stem Injections with *Peronospora
Tabacina* and Metalaxyl Treatment on Growth of Tobacco and
Protection Against Blue Mould in the Field,"
Phytopathology 74:804 (1984), which is hereby
incorporated by reference). These plants flowered
25   approximately 2-3 weeks earlier than control plants
(Tuzun, S., et al., "Movement of a Factor in Tobacco
Infected with *Peronospora Tabacina* Adam which
Systemically Protects Against Blue Mould," Physiological
Plant Pathology 26:321-30 (1985), which is hereby
30   incorporated by reference).

        Systemic protection does not confer absolute
immunity against infection, but reduces the severity of
the disease and delays symptom development. Lesion
number, lesion size, and extent of sporulation of fungal

pathogens are all decreased. The diseased area may be reduced by more than 90%.

When cucumbers were given a 'booster' inoculation 3-6 weeks after the initial inoculation, immunization induced by *C. lagenarium* lasted through flowering and fruiting (Kuc, J., et al., "Aspects of the Protection of Cucumber Against *Colletotrichum Lagenarium* by *Colletotrichum Lagenarium*," Phytopathology 67:533-6 (1977), which is hereby incorporated by reference). Protection could not be induced once plants had set fruit. Tobacco plants were immunized for the growing season by stem injection with sporangia of *P. tabacina*. However, to prevent systemic blue mould development, this technique was only effective when the plants were above 20 cm in height.

Removal of the inducer leaf from immunized cucumber plants did not reduce the level of immunization of pre-existing expanded leaves. However, leaves which subsequently emerged from the apical bud were progressively less protected than their predecessors (Dean, R.A., et al., "Induced Systemic Protection in Cucumber: Time of Production and Movement of the 'Signal'," Phytopathology 76:966-70 (1986), which is hereby incorporated by reference). Similar results were reported by Ross, A.F., "Systemic Effects of Local Lesion Formation," In: *Viruses of Plants* pp. 127-50 (1966), which is hereby incorporated by reference, with tobacco (local lesion host) immunized against tobacco mosaic virus by prior infection with tobacco mosaic virus. In contrast, new leaves which emerged from scions excised from tobacco plants immunized by stem-injection with *P. tabacina* were highly protected (Tuzun, S., et al., "Transfer of Induced Resistance in Tobacco to Blue Mould (Peronospora tabacina Adam.) Via Callus," Phytopathology 75:1304 (1985), which is hereby incorporated by

reference). Plants regenerated via tissue culture from leaves of immunized plants showed a significant reduction in blue mould compared to plants regenerated from leaves of non-immunized parents. Young regenerants only showed reduced sporulation. As plants aged, both lesion development and sporulation were reduced. Other investigators, however, did not reach the same conclusion, although a significant reduction in sporulation in one experiment was reported (Lucas, J.A., et al., "Nontransmissibility to Regenerants from Protected Tobacco Explants of Induced Resistance to *Peronospora Hyoscyami*," Phytopathology 75:1222-5 (1985), which is hereby incorporated by reference).

Protection of cucumber and watermelon is effective in the glasshouse and in the field (Caruso, F.L., et al., "Field Protection of Cucumber Against *Colletotrichum Lagenarium* by *C. Lagenarium*," Phytopathology 67:1290-2 (1977), which is hereby incorporated by reference). In one trial, the total lesion area of *C. lagenarium* on protected cucumber was less than 2% of the lesion areas on unprotected control plants. Similarly, only 1 of 66 protected, challenged plants died, whereas 47 of 69 unprotected, challenged watermelons died. In extensive field trials in Kentucky and Puerto Rico, stem injection of tobacco with sporangia of *P. tabacina* was at least as effective in controlling blue mould as the best fungicide, metalaxyl. Plants were protected, leading to a yield increase of 10-25% in cured tobacco.

Induced resistance against bacteria and viruses appears to be expressed as suppression of disease symptoms or pathogen multiplication or both (Caruso, F.L., et al., "Induced Resistance of Cucumber to Anthracnose and Angular Leaf Spot by *Pseudomonas Lachrymans* and *Colletotrichum Lagenarium*," Physiological

Plant Pathology 14:191-201 (1979); Doss, M., et al., "Systemic Acquired Resistance of Cucumber to *Pseudomonas Lachrymans* as Expressed in Suppression of Symptoms, but not in Multiplication of Bacteria," *Acta Phytopathologia Academiae Scientiarum Hungaricae* 16:(3-4), 269-72 (1981); Jenns, A.E., et al., "Non-Specific Resistance to Pathogens Induced Systemically by Local Infection of Cucumber with Tobacco Necrosis Virus, *Colletotrichum Lagenarium* or *Pseudomonas Lachrymans*," *Phytopathologia Mediterranea* 18:129-34 (1979), which are hereby incorporated by reference).

As described above, research concerning systemic acquired resistance involves infecting plants with infectious pathogens. Although studies in this area are useful in understanding how systemic acquired resistance works, eliciting such resistance with infectious agents is not commercially useful, because such plant-pathogen contact can weaken or kill plants. The present invention is directed to overcoming this deficiency.

SUMMARY OF THE INVENTION

The present invention relates to a method of producing plant seeds which impart pathogen resistance to plants grown from the seeds. This method involves applying a hypersensitive response elicitor polypeptide or protein in a non-infectious form to plant seeds under conditions where the polypeptide or protein contacts cells of the plant seeds.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plant seeds in order to impart pathogen resistance to plants grown from the seeds, transgenic seeds can be utilized. This involves providing a transgenic plant seed transformed

with a DNA molecule encoding a hypersensitive response
elicitor polypeptide or protein and planting that seed in
soil.   A plant is then propagated from the planted seed
under conditions effective to impart pathogen resistance

5   to the plant.

Another aspect of the present invention relates
to a pathogen-resistance imparting plant seed to which a
non-infectious hypersensitive response elicitor
polypeptide or protein has been applied.

10   The present invention has the potential to:
treat plant diseases which were previously untreatable;
treat diseases systemically that one would not want to
treat separately due to cost; and avoid the use of agents
that have an unpredictable effect on the environment and

15   even the plants.   The present invention can impart
resistance without using agents which are harmful to the
environment or pathogenic to the plant seeds being
treated or to plants situated near the location that
treated seeds are planted.   Since the present invention

20   involves use of a natural product that is fully and
rapidly biodegradable, the environment would not be
contaminated.

DETAILED DESCRIPTION OF THE INVENTION

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The present invention relates to a method of
producing plant seeds which impart pathogen resistance to
plants grown from the seeds.   This method involves
applying a hypersensitive response elicitor polypeptide

30   or protein in a non-infectious form to a plant seed under
conditions effective to impart disease resistance to a
plant grown from the seed.

As an alternative to applying a hypersensitive
response elicitor polypeptide or protein to plant seeds

35   in order to impart pathogen resistance to plants grown

from the seeds, transgenic seeds can be utilized. This involves providing a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and planting that seed in 5 soil. A plant is then propagated from the planted seed under conditions effective to impart pathogen resistance to the plant.

Another aspect of the present invention relates to a pathogen-resistance imparting plant seed to which a 10 non-infectious hypersensitive response elicitor polypeptide or protein has been applied.

The hypersensitive response elicitor polypeptide or protein utilized in the present invention can correspond to hypersensitive response elicitor 15 polypeptides or proteins derived from a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor.

Examples of suitable bacterial sources of 20 polypeptide or protein elicitors include *Erwinia*, *Pseudomonas*, and *Xanthamonas* species (e.g., the following bacteria: *Erwinia amylovora*, *Erwinia chrysanthemi*, *Erwinia stewartii*, *Erwinia carotovora*, *Pseudomonas syringae*, *Pseudomonas solancearum*, *Xanthomonas* 25 *campestris*, or mixtures thereof).

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of such fungal pathogens include *Phytophthora parasitica*, *Phytophthora* 30 *cryptogea*, *Phytophthora cinnamomi*, *Phytophthora capsici*, *Phytophthora megasperma*, and *Phytophthora citrophthora*.

The embodiment of the present invention where the hypersensitive response elicitor polypeptide or protein is applied to the plant seed can be carried out

in a number of ways, including: 1) application of an isolated elicitor polypeptide or protein; 2) application of bacteria which do not cause disease and are transformed with genes encoding a hypersensitive response

5   elicitor polypeptide or protein; and 3) application of bacteria which cause disease in some plant species (but not in those to which they are applied) and naturally contain a gene encoding the hypersensitive response elicitor polypeptide or protein. In addition, seeds in

10   accordance with the present invention can be recovered from plants which have been treated with a hypersensitive response elicitor protein or polypeptide in accordance with the present invention.

In one embodiment of the present invention,

15   the hypersensitive response elicitor polypeptides or proteins to be applied can be isolated from their corresponding organisms and applied to plants. Such isolation procedures are well known, as described in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet,

20   and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543 - 553 (1994); He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv.

25   *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993); and Wei, Z.-M., R. J. Laby, C. H. Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin Elicitor of the Hypersensitive

30   Response Produced by the Plant Pathogen *Erwinia amylovora*, Science 257:85-88 (1992), which are hereby incorporated by reference. See also pending U.S. Patent Application Serial Nos. 08/200,024 and 08/062,024, which are hereby incorporated by reference. Preferably,

35   however, the isolated hypersensitive response elicitor

polypeptides or proteins of the present invention are produced recombinantly and purified as described below.

In other embodiments of the present invention, the hypersensitive response elicitor polypeptide or protein of the present invention can be applied to plant seeds by applying bacteria containing genes encoding the hypersensitive response elicitor polypeptide or protein. Such bacteria must be capable of secreting or exporting the polypeptide or protein so that the elicitor can contact plant seed cells. In these embodiments, the hypersensitive response elicitor polypeptide or protein is produced by the bacteria after application to the seeds or just prior to introduction of the bacteria to the seeds.

In one embodiment of the bacterial application mode of the present invention, the bacteria to be applied do not cause the disease and have been transformed (e.g., recombinantly) with genes encoding a hypersensitive response elicitor polypeptide or protein. For example, *E. coli*, which do not elicit a hypersensitive response in plants, can be transformed with genes encoding a hypersensitive response elicitor polypeptide and other related proteins required for production and secretion of the elicitor which is then applied to plant seeds. Expression of this polypeptide or protein can then be caused to occur. Bacterial species (other than *E. coli*) can also be used in this embodiment of the present invention.

In another embodiment of the bacterial application mode of the present invention, the bacteria do cause disease and naturally contain a gene encoding a hypersensitive response elicitor polypeptide or protein. Examples of such bacteria are noted above. However, in this embodiment these bacteria are applied to plant seeds for plants which are not susceptible to the disease

carried by the bacteria. For example, *Erwinia amylovora* causes disease in apple or pear but not in tomato. However, such bacteria will elicit a hypersensitive response in tomato. Accordingly, in accordance with this embodiment of the present invention, *Erwinia amylovora* can be applied to tomato seeds to impart pathogen resistance without causing disease in plants of that species.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20              25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35              40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50              55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
            85              90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100             105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
            115             120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
        130             135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145             150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175

Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180             185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
            195             200                 205
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Leu	Ser	Asn	Val	Ser	Thr	His	Val	Asp	Gly	Asn	Asn	Arg	His	Phe	Val
	210					215				220			

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
　　 210　　　　　　　 215　　　　　 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225　　　　　 230　　　　　 235　　　　　 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
　　　　　 245　　　　　 250　　　　　 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
　　　 260　　　　　 265　　　　　 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
　　 275　　　　　 280　　　　　 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
　 290　　　　　 295　　　　　 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305　　　　　 310　　　　　 315　　　　　 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
　　　 325　　　　　 330　　　　　 335

Asn Ala

This hypersensitive response elicitor polypeptide or
protein has a molecular weight of 34 kDa, is heat stable,
has a glycine content of greater than 16%, and contains
substantially no cysteine. The *Erwinia chrysanthemi*
hypersensitive response elicitor polypeptide or protein
is encoded by a DNA molecule having a nucleotide sequence
corresponding to SEQ. ID. No. 2 as follows:

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CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG      60
GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC     120
GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG     180
CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG     240
TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG     300
CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG     360
ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC     420
CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT     480
CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG     540
GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA     600
AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC     660
```

```
TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT      720

GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT      780

GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC      840

TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA      900

TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC      960

CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC     1020

CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG     1080

CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT     1140

GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT     1200

GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA     1260

CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA     1320

TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA     1380

GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG     1440

CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA     1500

TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC     1560

GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA     1620

ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAGAGAC GGGGAAGCCT GTCTCTTTTC     1680

TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA     1740

ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC     1800

GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC     1860

CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG     1920

CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG     1980

GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC     2040

AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG     2100

GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T                        2141
```

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

```
Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
  1           5               10              15
```

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
        20   25   30

5   Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
          35   40   45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
        50   55   60

10   Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
      65   70   75   80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
          85   90   95

15   Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
          100   105   110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
          115   120   125

20   Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
          130   135   140

25   Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
      145   150   155   160

Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
             165   170   175

30   Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
            180   185   190

35   Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
            195   200   205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
        210   215   220

40   Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
      225   230   235   240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
            245   250   255

45   Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
            260   265   270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
          275   280   285

50   Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
          290   295   300

55   Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
      305   310   315   320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
            325   330   335

60   Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
          340   345   350

```
          Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
              355                 360                 365

          Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
  5           370                 375                 380

          Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
              385                 390                 395                 400

 10       Gly Ala Ala
```

This hypersensitive response elicitor polypeptide or
protein has a molecular weight of about 39 kDa, it has a
pI of approximately 4.3, and is heat stable at 100°C for
at least 10 minutes. This hypersensitive response
elicitor polypeptide or protein has substantially no
cysteine. The hypersensitive response elicitor
polypeptide or protein derived from *Erwinia amylovora* is
more fully described in Wei, Z.-M., R. J. Laby, C. H.
Zumoff, D. W. Bauer, S.-Y. He, A. Collmer, and S. V.
Beer, "Harpin, Elicitor of the Hypersensitive Response
Produced by the Plant Pathogen *Erwinia amylovora*,"
Science 257:85-88 (1992), which is hereby incorporated by
reference. The DNA molecule encoding this polypeptide or
protein has a nucleotide sequence corresponding to SEQ.
ID. No. 4 as follows:

```
 30   AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA      60

      GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT     120

      ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG     180
 35
      GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG     240

      GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG     300

 40   GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA     360

      GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA     420

      GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC     480
 45
      TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC     540

      CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG     600

 50   CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC     660
```

```
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG     720

CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC     780

GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG     840

TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT     900

ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG     960

GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC    1020

CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC    1080

AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC    1140

ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC    1200

GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA    1260

CTTGGCAAGC TGGGCGCGGC TTAAGCTT                                       1288
```

The hypersensitive response elicitor
polypeptide or protein derived from *Pseudomonas syringae*
has an amino acid sequence corresponding to SEQ. ID.
No. 5 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5               10              15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20              25              30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35              40              45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
        50              55              60

Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65              70              75                      80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85              90              95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100             105             110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115             120             125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
        130             135             140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145             150             155             160
```

- 22 -

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165   170   175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
                180   185   190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195   200   205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210   215   220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225   230   235   240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
            245   250   255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
        260   265   270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275   280   285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290   295   300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305   310   315   320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
            325   330   335

Asn Gln Ala Ala Ala
            340

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* Harpin$_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 6 as follows:

```
ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG        60

GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC       120

GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA       180

AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG CGGCGGTAT TGAGGATGTC        240

ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG       300

GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC       360

AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC       420

GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC       480

AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC       540

GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG       600

AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC       660

AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC       720

GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA       780

TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG       840

GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG       900

GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT       960

GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA      1020

GCCTGA                                                                 1026
```

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 7 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5               10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20              25              30

Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35              40              45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
        50              55              60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65              70              75              80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
            85              90              95
```

```
Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100             105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
            115             120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
        130             135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145             150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165             170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180             185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
            195             200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210             215                 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225             230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245             250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260             265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275             280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290             295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305             310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
            325             330                 335

Gln Ser Thr Ser Thr Gln Pro Met
            340
```

It is encoded by a DNA molecule having a nucleotide
sequence corresponding SEQ. ID. No. 8 as follows:

```
ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC    60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC   120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC   180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC   240
```

```
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC    300

GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA    360

GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG    420

GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC    480

GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC    540

GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT    600

GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC    660

GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC    720

CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG    780

ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC    840

GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT    900

GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC    960

GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG    1020

ACGCAGCCGA TGTAA                                                    1035
```

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* pv. *glycines* has an amino acid sequence corresponding to SEQ. ID. No. 9 as follows:

```
Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile Ile Ala Ile Leu Ala
1               5                   10                  15

Ala Ile Ala Leu Pro Ala Tyr Gln Asp Tyr
                20              25
```

This sequence is an amino terminal sequence having 26 residues only from the hypersensitive response elicitor polypeptide or protein of *Xanthomonas campestris* pv. *glycines*. It matches with fimbrial subunit proteins determined in other *Xanthomonas campestris* pathovars.

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 10 as follows:

```
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
 1               5                   10                  15
Leu Leu Ala Met
         20
```

Isolation of *Erwinia carotovora* hypersensitive response elicitor protein or polypeptide is described in Cai, et al., "The RsmA⁻ Mutants of *Erwinia carotovora* subsp. *carotova* Strain Ecc71 Overexpress $hrpN_{Ecc}$ and Elicit a Hypersensitive Reaction-Like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide for *Erwinia stewartii* is disclosed in Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact, July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophora parasitica*, *Phytophora cryptogea*, *Phytophora cinnamoni*, *Phytophora capsici*, *Phytophora megasperma*, and *Phytophora citrophthora* are described in Kamoun, et al., "Extracellular Protein Elicitors from *Phytophora*: Host-Specificity and

Induction of Resistance to Bacterial and Fungal
Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-
25 (1993), Ricci, et al., "Structure and Activity of
Proteins from Pathogenic Fungi *Phytophora* Eliciting

5   Necrosis and Acquired Resistance in Tobacco," Eur. J.
Biochem., 183:555-63 (1989), Ricci, et al., "Differential
Production of Parasiticein, an Elicitor of Necrosis and
Resistance in Tobacco by Isolates of *Phytophora*
paraticica," Plant Path., 41:298-307 (1992), Baillieul,

10 et al., "A New Elicitor of the Hypersensitive Response in
Tobacco: A Fungal Glycoprotein Elicits Cell Death,
Expression of Defense Genes, Production of Salicylic
Acid, and Induction of Systemic Acquired Resistance,"
Plant J., 8(4):551-60 (1995), and Bonnet, et al.,

15 "Acquired Resistance Triggered by Elicitins in Tobacco
and Other Plants," Eur. J. Plant Path., 102:181-92
(1996), which are hereby incorporated by reference.

The above elicitors are exemplary. Other
elicitors can be identified by growing fungi or bacteria

20 that elicit a hypersensitive response under which genes
encoding an elicitor are expressed. Cell-free
preparations from culture supernatants can be tested for
elicitor activity (i.e. local necrosis) by using them to
infiltrate appropriate plant tissues.

25 It is also possible to use fragments of the
above hypersensitive response elicitor polypeptides or
proteins as well as fragments of full length elicitors
from other pathogens, in the method of the present
invention.

30 Suitable fragments can be produced by several
means. In the first, subclones of the gene encoding a
known elicitor protein are produced by conventional
molecular genetic manipulation by subcloning gene
fragments. The subclones then are expressed *in vitro* or

35 *in vivo* in bacterial cells to yield a smaller protein or

a peptide that can be tested for elicitor activity
according to the procedure described below.

As an alternative, fragments of an elicitor
protein can be produced by digestion of a full-length

5   elicitor protein with proteolytic enzymes like
chymotrypsin or *Staphylococcus* proteinase A, or trypsin.
Different proteolytic enzymes are likely to cleave
elicitor proteins at different sites based on the amino
acid sequence of the elicitor protein. Some of the

10   fragments that result from proteolysis may be active
elicitors of resistance.

In another approach, based on knowledge of the
primary structure of the protein, fragments of the
elicitor protein gene may be synthesized by using the PCR

15   technique together with specific sets of primers chosen
to represent particular portions of the protein. These
then would be cloned into an appropriate vector for
increased expression of a truncated peptide or protein.

An example of a suitable fragment is the popAl

20   fragment of the hypersensitive response elicitor
polypeptide or protein from *Pseudomonas solanacearum*.
See Arlat, M., F. Van Gijsegem, J.C. Huet, J.C. Pemollet,
and C.A. Boucher, "PopAl, a Protein Which Induces a
Hypersensitive-like Response in Specific Petunia

25   Genotypes is Secreted via the Hrp Pathway of *Pseudomonas
solanacearum*," EMBO J. 13:543-53 (1994), which is hereby
incorporated by reference. As to *Erwinia amylovora*, a
suitable fragment can be, for example, either or both the
polypeptide extending between and including amino acids 1

30   and 98 of SEQ. ID. NO. 3 and the polypeptide extending
between and including amino acids 137 and 204 of SEQ. ID.
No. 3.

Variants may be made by, for example, the
deletion or addition of amino acids that have minimal

35   influence on the properties, secondary structure and

hydropathic nature of the polypeptide. For example, a
polypeptide may be conjugated to a signal (or leader)
sequence at the N-terminal end of the protein which co-
translationally or post-translationally directs transfer

5 of the protein. The polypeptide may also be conjugated
to a linker or other sequence for ease of synthesis,
purification or identification of the polypeptide.

The protein or polypeptide of the present
invention is preferably produced in purified form

10 (preferably at least about 60%, more preferably 80%,
pure) by conventional techniques. Typically, the protein
or polypeptide of the present invention is produced but
not secreted into the growth medium of recombinant _E._
coli. Alternatively, the protein or polypeptide of the

15 present invention is secreted into the growth medium. In
the case of unsecreted protein, to isolate the protein,
the _E. coli_ host cell carrying a recombinant plasmid is
propagated, homogenized, and the homogenate is
centrifuged to remove bacterial debris. The supernatant

20 is then subjected to heat treatment and the
hypersensitive response elicitor protein is separated by
centrifugation. The supernatant fraction containing the
polypeptide or protein of the present invention is
subjected to gel filtration in an appropriately sized

25 dextran or polyacrylamide column to separate the
proteins. If necessary, the protein fraction may be
further purified by ion exchange or HPLC.

Alternatively, the hypersensitive response
elicitor protein can be prepared by chemical synthesis

30 using conventional techniques.

The DNA molecule encoding the hypersensitive
response elicitor polypeptide or protein can be
incorporated in cells using conventional recombinant DNA
technology. Generally, this involves inserting the DNA

35 molecule into an expression system to which the DNA

molecule is heterologous (i.e. not normally present).
The heterologous DNA molecule is inserted into the
expression system or vector in proper sense orientation
and correct reading frame. The vector contains the
5   necessary elements for the transcription and translation
of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer,
which is hereby incorporated by reference, describes the
production of expression systems in the form of
10   recombinant plasmids using restriction enzyme cleavage
and ligation with DNA ligase. These recombinant plasmids
are then introduced by means of transformation and
replicated in unicellular cultures including procaryotic
organisms and eucaryotic cells grown in tissue culture.

15   Recombinant genes may also be introduced into
viruses, such as vaccina virus. Recombinant viruses can
be generated by transection of plasmids into cells
infected with virus.

Suitable vectors include, but are not limited
20   to, the following viral vectors such as lambda vector
system gt11, gt WES.tB, Charon 4, and plasmid vectors
such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9,
pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40,
pBluescript II SK +/- or KS +/- (see "Stratagene Cloning
25   Systems" Catalog (1993) from Stratagene, La Jolla, Calif,
which is hereby incorporated by reference), pQE, pIH821,
pGEX, pET series (see F.W. Studier et. al., "Use of T7
RNA Polymerase to Direct Expression of Cloned Genes,"
Gene Expression Technology vol. 185 (1990), which is
30   hereby incorporated by reference), and any derivatives
thereof. Recombinant molecules can be introduced into
cells via transformation, particularly transduction,
conjugation, mobilization, or electroporation. The DNA
sequences are cloned into the vector using standard
35   cloning procedures in the art, as described by Sambrook

et al., <u>Molecular Cloning: A Laboratory Manual</u>, Cold
Springs Laboratory, Cold Springs Harbor, New York (1989),
which is hereby incorporated by reference.

        A variety of host-vector systems may be
5   utilized to express the protein-encoding sequence(s).
Primarily, the vector system must be compatible with the
host cell used. Host-vector systems include but are not
limited to the following: bacteria transformed with
bacteriophage DNA, plasmid DNA, or cosmid DNA;
10   microorganisms such as yeast containing yeast vectors;
mammalian cell systems infected with virus (e.g.,
vaccinia virus, adenovirus, etc.); insect cell systems
infected with virus (e.g., baculovirus); and plant cells
infected by bacteria. The expression elements of these
15   vectors vary in their strength and specificities.
Depending upon the host-vector system utilized, any one
of a number of suitable transcription and translation
elements can be used.

        Different genetic signals and processing events
20   control many levels of gene expression (e.g., DNA
transcription and messenger RNA (mRNA) translation).

        Transcription of DNA is dependent upon the
presence of a promotor which is a DNA sequence that
directs the binding of RNA polymerase and thereby
25   promotes mRNA synthesis. The DNA sequences of eucaryotic
promotors differ from those of procaryotic promotors.
Furthermore, eucaryotic promotors and accompanying
genetic signals may not be recognized in or may not
function in a procaryotic system, and, further,
30   procaryotic promotors are not recognized and do not
function in eucaryotic cells.

        Similarly, translation of mRNA in procaryotes
depends upon the presence of the proper procaryotic
signals which differ from those of eucaryotes. Efficient
35   translation of mRNA in procaryotes requires a ribosome

binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the

5   protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and

10 Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong

15 promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in E. coli, its bacteriophages, or

20 plasmids, promotors such as the T7 phage promoter, lac promotor, trp promotor, recA promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to lacUV5, ompF, bla, lpp, and the like, may be used to direct high levels of

25 transcription of adjacent DNA segments. Additionally, a hybrid trp-lacUV5 (tac) promotor or other E. coli promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

30 Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted

35 DNA. For example, the lac operon is induced by the

addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required
5   for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector,
10   which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires a Shine-Dalgarno (SD) sequence about 7-9 bases 5' to the initiation codon (ATG)
15   to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli*
20   tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the
25   hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system.
30   Suitable host cells include, but are not limited to, bacteria, virus, yeast, mammalian cells, insect, plant, and the like.

The method of the present invention can be utilized to treat seeds for a wide variety of plants to
35   impart pathogen resistance to the plants. Suitable seeds

are for plants which are dicots and monocots. More
particularly, useful crop plants can include: rice,
wheat, barley, rye, oats, cotton, sunflower, canola,
peanut, corn, potato, sweet potato, bean, pea, chicory,
5   lettuce, endive, cabbage, cauliflower, broccoli, turnip,
radish, spinach, onion, garlic, eggplant, pepper, celery,
carrot, squash, pumpkin, zucchini, cucumber, apple, pear,
melon, strawberry, grape, raspberry, pineapple, soybean,
tobacco, tomato, sorghum, and sugarcane. Examples of
10   suitable ornamental plants are: rose, *Saintpaulia*,
petunia, *Pelargonium*, poinsettia, chrysanthemum,
carnation, and zinnia.

The method of imparting pathogen resistance to
plants in accordance with the present invention is useful
15   in imparting resistance to a wide variety of pathogens
including viruses, bacteria, and fungi.

Resistance, *inter alia*, to the following
viruses can be achieved by the method of the present
invention: Tobacco mosaic virus, cucumber mosaic virus,
20   potato x virus, potato y virus, and tomato mosaic virus.

Resistance, *inter alia*, to the following
bacteria can also be imparted to plants in accordance
with the present invention: *Pseudomonas solancearum*,
Pseudomonas syringae pv. tabaci, and *Xanthamonas*
25   *campestris pv. pelargonii*.

Plants can be made resistant, *inter alia*, to
the following fungi by use of the method of the present
invention: *Fusarium oxysporum* and *Phytophthora*
infestans.

30   The embodiment of the present invention
involving applying the hypersensitive response elicitor
polypeptide or protein to all or part of the plant seeds
being treated can be carried out through a variety of
procedures. Suitable application methods include high or
35   low pressure spraying, injection, coating, dusting, and

immersion. Other suitable application procedures can be envisioned by those skilled in the art. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to enhance hypersensitive response induced resistance in the plants. See U.S. Patent Application Serial No. 08/475,775, which is hereby incorporated by reference. Such propagated plants, which are resistant to disease, may, in turn, be useful in producing seeds or propagules (e.g. cuttings) that produce resistant plants.

The hypersensitive response elicitor polypeptide or protein can be applied to plant seeds in accordance with the present invention alone or in a mixture with other materials.

A composition suitable for treating plant seeds in accordance with the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 0.5 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematicide, herbicide, and mixtures thereof. Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the

process of the present invention. In addition, the
hypersensitive response elicitor polypeptide or protein
can be applied to plant seeds with other conventional
seed formulation and treatment materials, including clays
5   and polysaccharides.

In the alternative embodiment of the present
invention involving the use of transgenic seeds, a
hypersensitive response elicitor polypeptide or protein
need not be applied topically to the seeds. Instead,
10   transgenic plants transformed with a DNA molecule
encoding a hypersensitive response elicitor polypeptide
or protein are produced according to procedures well
known in the art, such as biolistics or *Agrobacterium*
mediated transformation. Examples of suitable
15   hypersensitive response elicitor polypeptides or proteins
and the nucleic acid sequences for their encoding DNA are
disclosed *supra*. As is conventional in the art, such
transgenic plants would contain suitable vectors with
various promoters including pathogen-induced promoters,
20   and other components needed for transformation,
transcription, and, possibly, translation. Such
transgenic plants themselves could be grown under
conditions effective to be imparted with pathogen
resistance. In any event, once transgenic plants of this
25   type are produced, transgenic seeds are recovered. These
seeds can then be planted in the soil and cultivated
using conventional procedures to produce plants. The
plants are propagated from the planted transgenic seeds
under conditions effective to impart pathogen resistance
30   to the plants.

When transgenic plant seeds are used in
accordance with the present invention, they additionally
can be treated with the same materials (noted above) as
are used to treat the seeds to which a hypersensitive
35   response elicitor polypeptide or protein is applied.

These other materials, including hypersensitive response
elicitors, can be applied to the transgenic plant seeds
by high or low pressure spraying, injection, coating,
dusting, and immersion. Similarly, transgenic plants

5   additionally may be treated with one or more applications
of the hypersensitive response elicitor to enhance
hypersensitive response induced resistance in the plants.
Such plants may also be treated with conventional plant
treatment agents (e.g., insecticides, fertilizers, etc.).

10   The transgenic plants of the present invention are useful
in producing seeds or propagules (e.g. cuttings) from
which disease resistant plants grow.

EXAMPLES

15

Example 1 - Effect of Treating Seeds with Hypersensitive Response Elicitor Protein

20   *Marglobe* tomato seeds were submerged in
hypersensitive response elicitor protein (ca. 26 μgm/ml)
from *Erwinia amylovora* solution or buffer in beakers on
day 0 for 24 hours at 28°C in a growth chamber. After
soaking seeds in hypersensitive response elicitor protein

25   from *Erwinia amylovora* or buffer, they were sown in
germination pots with artificial soil on day 1.
Seedlings were transplanted to individual pots at the
two-true-leaf stage on day 12. After transplanting, some
plants that arose from treated seed also were sprayed

30   with hypersensitive response elicitor protein (ca. 13
μgm/ml) from *Erwinia amylovora* (Treatments 3 and 4).
        Tomato treated as noted in the preceding
paragraph were inoculated with *Burkholderia (Pseudomonas)*
solanacearum K60 strain (See Kelman, "The Relationship of

35   Pathogenicity in *Pseudomonas solanacearum* to Colony
Appearance on a Tetrazolium Medium," Phytopathology
44:693-95 (1954)) on day 23 by making vertical cuts

through the roots and potting medium of tomato plants (on a tangent 2 cm from the stem and 2 times/pot) and putting 10 ml (5 X 10^8cfu/ml) suspension into the soil.

The above procedure involved use of 10 seeds treated with hypersensitive response elicitor protein from *Erwinia amylovora* per treatment.

Treatments:

1. Seeds soaked in hypersensitive response elicitor protein from *Erwinia amylovora* (ca. 26 μmg/ml).

2. Seeds soaked in buffer (5mM KPO_4, pH 6.8).

3. Seeds soaked in hypersensitive response elicitor protein from *Erwinia amylovora* (ca. 26 μmg/ml) and seedlings sprayed with hypersensitive response elicitor protein from *Erwinia amylovora* (ca. 13 μgm/ml) at transplanting.

4. Seeds soaked in buffer and seedlings sprayed with hypersensitive response elicitor protein from *Erwinia amylovora* (ca. 13 μgm/ml) at transplanting.

The results of these treatments are set forth in Tables 1-4.

Table 1 - Infection Data - 28 Days After Seed Treatment and 5 Days After Inoculation

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	10	0	0	0	0	0
2	10	9	1	0	0	0	0
3	10	9	1	0	0	0	0
4	10	10	0	0	0	0	0

* **Disease Scale:**

Grade 0: No symptoms
Grade 1: One leaf partially wilted.
Grade 2: 2-3 leaves wilted.
Grade 3: All except the top 2-3 leaves wilted.
Grade 4: All leaves wilted.
Grade 5: Plant Dead

Table 2 - Infection Data - 31 Days After Seed Treatment and 8 Days After Inoculation

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	6	4	0	0	0	0
2	10	4	3	2	1	0	0
3	10	8	2	0	0	0	0
4	10	7	2	1	0	0	0

Table 3 - Infection Data - 35 Days After Seed
Treatment and 12 Days After Inoculation

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	5	3	0	1	1	0
2	10	1	3	3	2	1	0
3	10	4	3	3	0	0	0
4	10	3	3	3	1	0	0

Table 4 - Disease Indices of Seed Treatment
With Hypersensitive Response Elicitor Protein

Treatment		Inoculation	Disease Index (%)*		
Day 0	Day 14	Day 23	Day 28	Day 31	Day 35
1. Hypersensitive response elicitor protein seed soak		Inoculate	0	8	20
2. Buffer seed soak		Inoculate	2	20	38
3. Hypersensitive response elicitor protein seed soak	Spray Hypersensitive response elicitor protein	Inoculate	2	4	18
4. Buffer seed soak	Spray Hypersensitive response elicitor protein	Inoculate	0	8	24

* The Disease Index was determined using the procedure set forth in N.N. Winstead, et al., "Inoculation Techniques for Evaluating Resistance to *Pseudomonas Solanacearum*," Phytopathology 42:628-34 (1952), particularly at page 629.

The above data shows that the hypersensitive response elicitor protein was more effective than buffer as a seed treatment in reducing disease index and was as effective as spraying leaves of young plants with
5   *hypersensitive response elicitor protein.*

Example 2 -   **Effect of Treating Tomato Seeds With Hypersensitive Response Elicitor Protein From pCPP2139 Versus pCPP50 Vector On**
10   **Southern Bacteria Wilt Of Tomato**

Marglobe tomato seeds were submerged in hypersensitive response elicitor protein from pCPP2139 or in pCPP50 vector solution (1:50, 1:100 and 1:200) in
15   beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking seeds in hypersensitive response elicitor protein or vector, they were sown in germination pots with artificial soil on day 0. Ten uniform appearing plants were chosen randomly from each of the
20   following treatments:

Treatment Content	Strain	Dilution	Harpin
25 1.	DH5α(pCPP2139)	1:50	8 μg/ml
2.	DH5α(pCCP50)	1:50	0
3.	DH5α(pCPP2139)	1:100	4 μg/ml
4.	DH5α(pCPP50)	1:100	0
5.	DH5α(pCPP2139)	1:200	2 μg/ml
30 6.	DH5α(pCPP50)	1:200	0

The resulting seedlings were inoculated with *Pseudomonas*
35   *solanacearum* K60 by dipping the roots of tomato seedling plants for about 30 seconds in a 40 ml (1 X 10^8 cfu/ml) suspension. The seedlings were then transplanted into the pots with artificial soil on day 12.
         The results of these treatments are set forth
40   in Tables 5-8.

**Table 5 - 16 Days After Seed Treatment and
3 Days After Inoculation**

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	7	3	0	0	0	0
2	10	5	5	0	0	0	0
3	10	6	4	0	0	0	0
4	10	6	4	0	0	0	0
5	10	7	4	0	0	0	0
6	10	4	6	0	0	0	0

**Table 6 - 19 Days After Seed Treatment and
6 Days After Inoculation**

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	6	0	0	0	0	0
2	10	2	0	2	2	1	3
3	10	2	0	2	0	2	4
4	10	3	1	2	0	2	2
5	10	2	1	0	2	2	3
6	10	1	0	1	1	3	4

Table 7 - 21 Days After Seed Treatment and 8 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	6	0	0	0	1	3
2	10	2	0	0	1	3	4
3	10	2	0	0	2	2	3
4	10	3	0	0	2	2	3
5	10	2	0	0	0	4	4
6	10	1	0	1	2	1	5

Table 8 - Disease Indices of Seed Treatment With Hypersensitive Response Elicitor and Vector

Treatment		Disease Index (%)		
Day 0	Day 12	Day 15	Day 18	Day 20
Hypersensitive response elicitor protein seed dip (1:50)	inoculate	6.0	32.0	38.0
Vector seed dip (1:50)	inoculate	10.0	58.0	70.0
Hypersensitive response elicitor protein seed dip (1:100)	inoculate	8.0	64.0	68.0
Vector seed dip (1:100)	inoculate	8.0	46.0	58.0
Hypersensitive response elicitor protein seed dip (1:200)	inoculate	6.0	60.00	72.0
Vector seed dip (1:200)	inoculate	12.0	74.0	74.0

The above data shows that the hypersensitive response elicitor protein is much more effective than the vector solution in preventing Tomato Southern Bacteria Wilt.

- 44 -

Example 3 -   **Effect of Treating Tomato Seeds With Hypersensitive Response Elicitor Protein From pCPP2139 Versus pCPP50 Vector On Tomato Southern Bacteria Wilt**

Marglobe tomato seeds were submerged in hypersensitive response elicitor protein from pCPP2139 or in pCPP50 vector solution (1:50, 1:100 and 1:200) in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking seeds in the hypersensitive response elicitor protein or vector, the seeds were sown in germination pots with artificial soil on day 1. Ten uniform appearing plants were chosen randomly from each of the following treatments:

Treatment	Strain	Dilution	Hypersensitive Response Elicitor Content
1.	DH5α(pCPP2139)	1:50	8 μg/ml
2.	DH5α(pCCP50)	1:50	0
3.	DH5α(pCPP2139)	1:100	4 μg/ml
4.	DH5α(pCPP50)	1:100	0
5.	DH5α(pCPP2139)	1:200	2 μg/ml
6.	DH5α(pCPP50)	1:200	0

The resulting seedlings were inoculated with *Pseudomonas solanacearum* K60 by dipping the roots of tomato seedling plants for about 30 seconds in a 40 ml (1 X 10^6 cfu/ml) suspension. The seedlings were then transplanted into the pots with artificial soil on day 12.

The results of these treatments are set forth in Tables 9-12.

Table 9 - 16 Days After Seed Treatment and 3 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	8	2	0	0	0	0
2	10	7	3	0	0	0	0
3	10	7	3	0	0	0	0
3	10	7	3	0	0	0	0
5	10	8	2	0	0	0	0
6	10	7	3	0	0	0	0

Table 10 - 19 Days After Seed Treatment and 6 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	5	0	0	1	2	2
2	10	1	0	1	2	3	3
3	10	3	1	0	0	2	3
4	10	2	0	2	1	2	3
5	10	1	0	1	1	4	3
6	10	1	0	0	2	4	3

Table 11 - 21 Days After Hypersensitive Response Elicitor Protein Seed Treatment and 8 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	5	0	0	0	2	3
2	10	2	0	2	0	2	4
3	10	5	0	0	0	2	3
4	10	2	0	2	0	2	4
5	10	1	0	1	0	2	6
6	10	1	0	0	0	2	7

Table 12 - Disease Indices of Seed Treatment With Hypersensitive Response Elicitor Protein and Vector

Day 1	Day 13	Day 16	Day 19	Day 21
Hypersensitive response elicitor protein seed dip (1:50)	inoculate	4.0	42.0	46.0
Vector seed dip (1:50)	inoculate	6.0	70.0	64.0
Hypersensitive response elicitor protein seed dip (1:100)	inoculate	6.0	48.0	46.0
Vector seed dip (1:100)	inoculate	6.0	60.0	64.0
Hypersensitive response elicitor protein seed dip (1:200)	inoculate	4.0	72.0	80.0
Vector seed dip (1:200)	inoculate	6.0	74.0	86.0

The above data shows that the hypersensitive response elicitor protein is much more effective in preventing Tomato Southern Bacteria Wilt.

Example 4 -   Effect of Treating Tomato Seeds With
              Hypersensitive Response Elicitor Protein
              From pCPP2139 Versus pCPP50 Vector On
              Southern Bacteria Wilt Of Tomato

Marglobe tomato seeds were submerged in
hypersensitive response elicitor protein from pCPP2139 or
in pCPP50 vector solution (1:25, 1:50 and 1:100) in
beakers on day 0 for 24 hours at 28°C in a growth
chamber. After soaking seeds in hypersensitive response
elicitor protein or vector, they were sown in germination
pots with artificial soil on day 1. Ten uniform
appearing plants were chosen randomly from each of the
following treatments:

Treatment Content	Strain	Dilution	Harpin
1.	DH5α(pCPP2139)	1:25	16 μg/ml
2.	DH5α(pCCP50)	1:25	0
3.	DH5α(pCPP2139)	1:50	8 μg/ml
4.	DH5α(pCPP50)	1:50	0
5.	DH5α(pCPP2139)	1:100	2 μg/ml
6.	DH5α(pCPP50)	1:100	0

The resulting seedlings were inoculated with *Pseudomonas
solanacearum* K60 by dipping the roots of tomato seedling
plants for about 30 seconds in a 40 ml (1×10^8 cfu/ml)
suspension. The seedlings were then transplanted into
the pots with artificial soil on day 14.

The results of these treatments are set forth
in Tables 13-16.

**Table 13 - 19 Days After Seed Treatment and
4 Days After Inoculation**

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	8	2	0	0	0	0
2	10	5	2	2	1	0	0
3	10	9	1	0	0	0	0
4	10	5	2	1	●	0	0
5	10	5	3	1	1	0	0
6	10	6	1	2	1	0	0

**Table 14 - 21 Days After Seed Treatments and
6 Days After Inoculation**

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
4	10	6	3	0	0	1	0
2	10	3	2	1	0	0	0
3	10	6	3	1	0	0	0
4	10	3	2	1	2	2	0
5	10	5	1	2	2	0	0
6	10	3	1	3	2	1	0

Table 15 - 23 Days After Seed Treatment and
8 Days After Inoculation

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	7	2	0	0	0	1
2	10	2	2	2	3	0	1
3	10	7	2	0	1	0	0
4	10	2	1	2	3	0	2
5	10	3	1	2	3	0	1
6	10	2	2	2	3	0	1

Table 16 - Disease Indices of Seed Treatment
With Hypersensitive Elicitor Protein and Vector

Treatment		Disease Index (%)		
Day 1	Day 15	Day 19	Day 21	Day 23
Hypersensitive response elicitor protein seed dip (1:25)	inoculate	4.0	14.0	14.0
Vector seed dip (1:25)	inoculate	18.0	28.0	40.0
Hypersensitive response elicitor protein seed dip (1:50)	inoculate	2.0	10.0	10.0
Vector seed dip (1:50)	inoculate	20.0	36.0	48.0
Hypersensitive response elicitor protein seed dip (1:100)	inoculate	16.0	22.0	38.0
Vector seed dip (1:100)	inoculate	16.0	34.0	40.0

The above data shows that the hypersensitive response elicitor protein is much more effective than the vector solution in preventing Tomato Southern Bacteria

Wilt. A hypersensitive response protein concentration of 1:50 is particularly effective in disease control.

5   **Example 5** -   **Effect of Treating Tomato Seeds With Hypersensitive Response Elicitor Protein From pCPP2139 Versus pCPP50 Vector On Southern Bacteria Wilt Of Tomato**

         Marglobe tomato seeds were submerged in
10 hypersensitive response elicitor protein from pCPP2139 or pCPP50 vector solution (1:25, 1:50 and 1:100) in beakers on day 0 for 24 hours at 28°C in a growth chamber. After soaking seeds in hypersensitive response elicitor protein or vector, they were sown in germination pots with
15 artificial soil on day 1. Ten uniform appearing plants were chosen randomly from each of the following treatments:

Treatment Content	Strain	Dilution	Harpin
1.	DH5α(pCPP2139)	1:25	16 µg/ml
2.	DH5α(pCCP50)	1:25	0
3.	DH5α(pCPP2139)	1:50	8 µg/ml
4.	DH5α(pCPP50)	1:50	0
5.	DH5α(pCPP2139)	1:100	4 µg/ml
6.	DH5α(pCPP50)	1:100	0

30 The resulting seedlings were inoculated with *Pseudomonas solanacearum* K60 by dipping the roots of tomato seedling plants for about 30 seconds in a 40 ml (1 X 10^6 cfu/ml) suspension. The seedlings were then transplanted into the pots with artificial soil on day 14.
35   The results of these treatments are set forth in Tables 17-20.

Table 17 - 19 Days After Seed Treatment and
4 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	8	2	0	0	0	0
2	10	6	3	1	0	0	0
3	10	9	1	0	0	0	0
4	10	6	4	0	0	0	0
5	10	6	2	1	1	0	0
6	10	6	4	0	0	0	0

Table 18 - 21 Days After Seed Treatment and
6 Days After Inoculation

Treatm.	Plants	Number of Plants of Given Disease Rating*					
		0	1	2	3	4	5
1	10	7	1	1	1	0	0
2	10	3	3	2	2	0	0
3	10	8	2	0	0	0	0
4	10	3	3	2	2	0	0
5	10	6	1	1	2	0	0
6	10	3	2	3	1	1	0

Table 19 - 23 Days After Seed Treatment and
8 Days After Inoculation

		Number of Plants of Given Disease Rating*					
Treatm.	Plants	0	1	2	3	4	5
1	10	7	0	2	1	0	0
2	10	3	1	2	3	0	1
3	10	8	1	0	1	0	0
4	10	3	3	1	2	0	1
5	10	3	3	0	2	1	1
6	10	3	2	0	3	0	2

Table 20 - Disease Indices of Seed Treatment
With Hypersensitive Response Elicitor Protein and Vector

Treatment		Disease Index (%)		
Day 0	Day 15	Day 19	Day 21	Day 23
Hypersensitive response elicitor protein seed dip (1:25)	inoculate	4.0	12.0	14.0
Vector seed dip (1:25)	inoculate	10.0	26.0	38.0
Hypersensitive response elicitor protein seed dip (1:50)	inoculate	2.0	4.0	8.0
Vector seed dip (1:50)	inoculate	8.0	26.0	32.0
Hypersensitive response elicitor protein seed dip (1:100)	inoculate	14.0	18.0	36.0
Vector seed dip (1:100)	inoculate	8.0	30.0	42.0

The above data shows that the hypersensitive response elicitor protein is much more effective than the

- 53 -

vector solution in preventing Tomato Southern Bacteria
Wilt. A hypersensitive response elicitor protein
concentration of 1:50 is more effective in disease
control.

5

Example 6 -   Treating Rice Seeds with Hypersensitive
                  Response Elicitor Protein to Reduce Rice
                  Stem Rot

10   Rice seeds (variety, M-202) were submerged in
two gallons of hypersensitive response elicitor protein
solution at a concentration of 20 μg for 24 hours at room
temperature. Rice seeds submerged in the same solution
without hypersensitive response elicitor protein were
15 used as a control. After soaking, the seeds were sown in
a rice field by air plane spray. There were four
replicates for both hypersensitive response elicitor
protein and control treatment. The lot size of each
replicate is 150 Ft². The design of each plot was
20 completely randomized, and each plot had substantial
level contamination of *Sclerotium oryzae*. Three months
after sowing, stem rot was evaluated according to the
following rating scale: Scale 1 = no disease, 2 =
disease present on the exterior of the leaf sheath, 3 =
25 disease penetrates leaf sheath completely but is not
present on culm, 4 = disease is present on culm exterior
but does not penetrate to interior of culm, and 5 =
disease penetrates to interior of culm. 40 plants from
each replicate were sampled and assessed for the disease
30 incidence and severity. From Table 21, it is apparent
that treating seeds with hypersensitive response elicitor
reduced both disease incidence and severity. More
particularly, regarding incidence, 67% of the plants were
infected by stem rot for the control treatment, however,
35 only 40% plants were infected for the hypersensitive
response elicitor protein treatment. As to severity, the
disease index* for the hypersensitive response elicitor

protein treatment was 34% and 60% for the control. Accordingly, treating rice seed with hypersensitive response elicitor protein resulted in a significant reduction of stem rot disease. The hypersensitive

5   response elicitor protein-induced resistance in rice can last a season long. In addition to disease resistance, it was also observed that hypersensitive response elicitor protein-treated rice had little or no damage by army worm (*Spodoptera praefica*). In addition, the

10   treated plants were larger and had deeper green color than the control plants.

Table 21 - Incidence and Severity of Stem Rot (*Schlerotium oryzae*) on Rice, M-202

15

Treatment	% plants given disease rating					Disease index(%) (severity)
	1	2	3	4	5	
Harpin 20 μg/ml	60	5	8	18	10	34
Control	33	5	18	28	18	60

20

*Disease Index (%) for the harpin treatment

25   $$= \frac{1\times60 + 2\times5 + 3\times8 + 4\times18 + 5\times10}{5\times100} \times 100/100$$

30   *Disease Index (%) for the control treatment

$$= \frac{1\times33 + 2\times5 + 3\times18 + 4\times28 + 5\times18}{5\times100\times100/100} \times 100/100$$

35

Example 7 -   Effect of Treating Onion Seed with
                Hypersensitive Response Elicitor Protein
                on the Development of Onion Smut Disease
                (*Urocystis cepulae*) and On Seedling
5   Emergence

            Onion seed, variety Pennant, (Seed Lot# 64387),
obtained from the Crookham Co., Caldwell, ID 83606,
10   treated with hypersensitive response elicitor protein or
a control was planted in a natural organic or "muck"
soil. Some of the seedlings that grew from the sown seed
were healthy, some had lesions characteristic of the
Onion Smut disease, and some of the sown seed did not
15   produce seedlings that emerged from the soil. Thus, the
effect of treating onion seed with various concentrations
of hypersensitive response elicitor protein was
determined.
            Naturally infested muck soil was obtained from
20   a field in Oswego County, NY, where onions had been grown
for several years and where the Onion Smut disease
commonly had been problematic. Buckets of muck (5-gallon
plastic) were stored at 4°C until used. The soil was
mixed, sieved, and put in plastic flats 10 inches wide,
25   20 inches long, and 2 inches deep for use in the tests
described. Based on preliminary experiments, the soil
contained many propagules of the Onion Smut fungus,
Urocystis cepulae, such that when onion seed was sown in
the soil, smut lesions developed on many of the seedlings
30   that emerged from the soil. In addition, the soil
harbored other microorganisms, including those that cause
the "damping-off" disease. Among the several fungi that
cause damping off are *Pythium, Fusarium,* and *Rhizoctonia*
species.
35   The hypersensitive response elicitor protein
encoded by the *hrpN* gene of *Erwinia amylovora* was used to
treat seeds. It was produced by fermentation of the
cloned gene in a high-expression vector in *E. coli.*
Analysis of the cell-free elicitor preparation by high-

pressure liquid chromatography indicated its hypersensitive response elicitor protein content and on that basis appropriate dilutions were prepared in water. Seeds were soaked in a beaker containing hypersensitive

5   response elicitor protein concentrations of 0, 5, 25, and 50 μgm/ml of hypersensitive response elicitor protein for 24 hours. They were removed, dried briefly on paper towels, and sown in the muck soil. Treated seed was arranged by row, 15 seeds in each row for each treatment;

10   each flat contained two replicates, and there were six replicates. Thus, a total of 90 seeds were treated with each concentration of hypersensitive response elicitor protein. The flats containing the seeds were held in a controlled environment chamber operating at 60°F

15   (15.6°C), with a 14-hour day /10-hour night. Observations were made on seedling emergence symptoms (smut lesions). The data were recorded 23 days after sowing.

The effect of soaking onion seed in different concentrations of hypersensitive response elicitor

20   protein on emergence of onion seedlings and on the incidence of onion smut is shown in Table 22. Only slight differences in emergence were noted, suggesting that there is no significant effect of treating with hypersensitive response elicitor protein at the

25   concentrations used. Among the seedlings that emerged, substantially more of the seeds that received no hypersensitive response elicitor protein exhibited symptoms of Onion Smut than seedlings that grew from seed that had been treated with hypersensitive response

30   elicitor protein. Treating seed with 25 μgm/ml of hypersensitive response elicitor protein was the most effective concentration tested in reducing Onion Smut. Thus, this example demonstrates that treating onion seed with hypersensitive response elicitor protein reduces the

35   Onion Smut disease.

Table 22 - Effect of Treating Onion Seed With Hypersensitive Response Elicitor Protein (i.e. Harpin) on the Development of Onion Smut Disease (*Urocystis cepulae*).

Treatment harpin (µg/ml)	Mean Seedlings Emerged (of 15)	Mean Percent Emerged	Emerged	
			Percent Healthy	Percent with Smut
0	5.00	33.3	20.0	80.0
5	3.67	24.4	40.9	59.1
25	4.33[1]	28.8	50.0	46.2
50	4.17	27.7	44.0	56.0

[1] One seedling emerged then died.

Example 8 - Effect of Treating Tomato Seed with Hypersensitive Response Elicitor Protein on the Development of Bacterial Speck of Tomato (*Pseudomonas syringae* pv. *tomato*)

Tomato seed, variety New Yorker (Seed lot# 2273-2B), obtained from Harris Seeds, Rochester, NY, were treated with four concentrations of hypersensitive response elicitor protein (including a no-elicitor protein, water-treated control) and planted in peatlite soil mix. After 12 days and when the seedlings were in the second true-leaf stage, they were inoculated with the Bacterial Speck pathogen. Ten days later, the treated and inoculated plants were evaluated for extent of infection. Thus, the effect of treating tomato seed with various concentrations of hypersensitive response elicitor protein on resistance to *Pseudomonas syringae* pv. *tomato* was determined.

The hypersensitive response elicitor protein encoded by the *hrpN* gene of *Erwinia amylovora* was used to treat seeds. It was produced by fermentation of the cloned gene in a high-expression vector in *E. coli*.

Analysis of the cell-free elicitor preparation by high-pressure liquid chromatography indicated its hypersensitive response elicitor protein content and, on that basis, appropriate dilutions were prepared in water.

5 Seeds were soaked in a beaker containing hypersensitive response elicitor protein concentrations of 0, 5, 10, and 20 μgm/ml of hypersensitive response elicitor protein for 24 hours. They were removed, dried briefly on paper towels, and sown. The soil was a mixture of peat and

10 Pearlite™ in plastic flats 10 inches wide, 20 inches long, and 2 inches deep. Treated seed was arranged by row, 6 seeds in each row for each treatment; each flat contained two replicates, and there were four replicates and thus a total of 24 seeds that were treated with each

15 concentration of hypersensitive response elicitor protein. The flats containing the seeds were held in a controlled environment chamber operating at 75°F (25°C), with a 14-hour day/10-hour night.

When twelve-days old, the tomato seedlings were

20 inoculated with 10^8 colony forming units/ml of the pathogen, applied as a foliar spray. The flats containing the seedlings were covered with a plastic dome for 48 hours after inoculation to maintain high humidity. Observations were made on symptom severity using a

25 rating scale of 0-5. The rating was based on the number of lesions that developed on the leaflets and the cotyledons and on the relative damage caused to the plant parts by necrosis that accompanied the lesions. The cotyledons and (true) leaflets were separately rated for

30 disease severity 11 days after inoculation

The effect of soaking tomato seed in different concentrations of hypersensitive response elicitor protein (i.e. harpin) on the development of Bacterial Speck on leaflets and cotyledons of tomato is shown in

35 Table 23. The seedlings that grew from seed treated with the highest amount of hypersensitive response elicitor

protein tested (20 μgm/ml) had fewer diseased leaflets and cotyledons than the treatments. The water-treated control seedlings did not differ substantially from the plants treated with the two lower concentrations of

5 hypersensitive response elicitor protein. Considering the disease ratings, the results were similar. Only plants treated with the highest concentration of hypersensitive response elicitor protein had disease ratings that were less than those of the other

10 treatments. This example demonsrates that treatment of tomato seed with hypersensitive response elicitor protein reduces the incidence and severity of Bacterial Speck of tomato.

15 **Table 23 - Effect of Treating Tomato Seed With Hypersensitive Response Elicitor Protein (i.e. Harpin) on the Subsequent Development of Bacterial Speck Disease (*Pseudomonas syringae* pv. tomato) on Tomato Cotyledons and Tomato Leaflets**

20

Treatment Harpin (μg/ml)	Cotyledons			Leaflets		
	Mean Diseased	Percent Diseased	Disease Rating	Mean Diseased	Percent Diseased	Disease Rating
0	6.0/9.0	66.6	0.8	25.8/68.8	37.5	0.5
5	5.3/7.3	72.4	0.8	22.5/68.0	37.5	0.5
10	5.8/8.0	72.3	0.8	25.5/66.0	38.6	0.5
20	5.3/8.5	61.8	0.6	23.8/73.5	32.3	0.4

Although the invention has been described in detail for the purpose of illustration, it is understood

35 that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.